PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Rama Ranganathan et al.

Serial No.: 09/684,066

Filed: October 6, 2000

For: STATISTICAL METHODS FOR ANALYZING BIOLOGICAL SEQUENCES

Group Art Unit: 1361

Examiner: Clow, L.

Atty. Dkt. No.: UTSD:645/MTG

SECOND DECLARATION OF RAMA RANGANATHAN

I, Rama Ranganathan, declare under penalty of perjury that:

1. I am over 18 years old and have personal knowledge of the facts below.

2. I am an Associate Professor at the University of Texas Southwestern Medical Center. I have held the title of Associate Professor for 2 years. I was an Assistant Professor for the preceding four years. I am also an Associate Investigator of the Howard Hughes Medical Institute, and have held that position for 2 years. I was an Assistant Investigator for the preceding four years.

3. In both capacities, 90% of my time is devoted to biological research. The remaining 10% of my time is devoted to teaching and other academic administrative duties.

4. I received my BS in engineering from Berkeley, and my MD and PhD degrees from the University of California, San Diego.

5. I am an inventor of this patent application.

6. Claims 1 and 10 include the step "accessing data representing a multiple sequence alignment (MSA) of a plurality of polymer sequences." The claimed accessing pertains to data that exists (*e.g.*, now or at some point in the future).

7. Claim 1 also includes the step "identifying one or more evolutionarily conserved amino acid positions within the MSA" using the equation that is provided. As a result of use of the term "amino acid positions," the claimed MSA concerns a polymer that includes one or more amino acid positions.

8. Claim 10 includes the steps "calculating a conservation energy value for each position in the MSA" using the recited equation and "identifying one or more positions within the MSA that have statistically significant conservation energy values."

9. The equation specified in claims 1 and 10 concerns certain probability parameters that are defined in each claim (P_i^x and P_{MSA}^x). P_i^x and P_{MSA}^x can be determined (*e.g.*, calculated) by using equation 2 on page 18 of the specification.

10. For claim 1, provided the probability parameters specified in the equation are known (*e.g.* through calculation), and the MSA concerns a polymer that includes one or more amino acid positions, the claimed identification can necessarily be achieved when the claimed equation is used.

11. For claim 10, provided the probability parameters specified in the equation are known (*e.g.* through calculation), the claimed identification can necessarily be achieved if the claimed equation is used.

12. The art to which the inventions defined by claims 1 and 10 pertain is bioinformatics that involves the use of an equation based on known/determinable parameters. In essence, claims 1-18 of our application concern mathematics, which is a well-established art.

13. The level of skill in this art is high because individuals working or researching in the bioinformatics field generally possess a masters or doctorate degree.

14. The predictability of this art (which I understand refers to the ability of someone of ordinary skill in this art to extrapolate the information provided in our application of how to use the claimed methods to other applications encompassed by our methods) is also high. This follows because once the parameters specified in the recited equation are known (*e.g.*, through calculation), the claimed identification can necessarily be achieved. For claim 10, this is true regardless of the type of polymer involved; and for claim 1, this is true regardless of the type of polymer involved, provided the polymer has one or more amino acid positions.

15. To illustrate the predictability of this art, and the applicability of the methods recited in claims 1 and 10 to MSAs of polymers other than those from the PDZ domain family, I have applied a method that can be used to fulfill the steps of claim 1 (if one or more evolutionarily conserved amino acid positions are identified) or claim 10 (if a conservation energy value for each position in the MSA is calculated, and one or more positions with statistically significant conservation energy values are identified) to an arbitrary model MSA of a made-up polymer.

16. I created an arbitrary twelve-residue model polymer made up of five different types of monomers. For simplicity, I identified the monomers as (1) circle, (2) spade, (3) heart, (4) diamond, and (5) club. The mean frequencies of the monomers in the universe of all known such polymers are known (I assumed them), and they are: 0.3 (circle), 0.2 (spade),

0.22 (heart), 0.11 (diamond), and 0.17 (club). One representative sequence of this polymer could be:

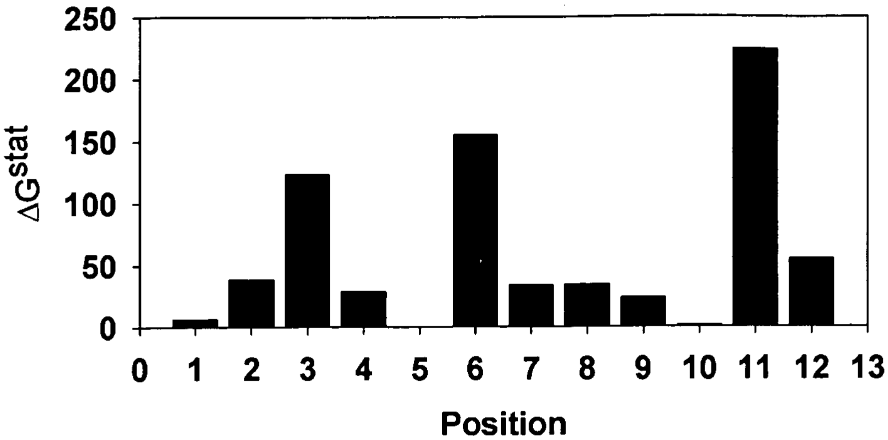
● – ♠ – ● – ♣ – ♥ – ♥ – ♦ – ● – ♥ – ♣ – ♦ – ♣

17. As described on page 18 of our application, each position in the MSA may be characterized by the frequencies of the five monomers, as given in the table below, which reflects values that were generated by computer to correspond to a multiple sequence alignment (MSA) that I assumed comprised 100 sequences of my model polymer. Each frequency is the count of each monomer at each position divided by the total number of sequences (100). The table below provides both the frequencies of each monomer at each site and the overall frequencies of each monomer in the MSA.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	*MSA overall*
Circle	0.35	0	1.0	0.3	0.33	0	0.33	0.5	0.33	0.25	0	0	0.2825
Spade	0.1	0.4	0	0.3	0.18	0	0.33	0	0	0.2	0	0.5	0.1675
Heart	0.2	0.21	0	0	0.2	1.0	0	0.5	0.33	0.25	0	0	0.2242
Diamond	0.05	0	0	0	0.14	0	0.34	0	0	0.08	1.0	0	0.1342
Club	0.3	0.39	0	0	0.15	0	0	0	0.34	0.22	0	0.5	0.1727

18. The next step is conversion of the frequencies at at least one MSA position (and, in this case, each MSA position) and in the MSA overall into binomial probabilities (P_i^x and P_{MSA}^x, respectively) using Equation 2 on page 18 of our application, given the mean frequency of that MSA position (and, in this case, each MSA position) in all known polymers (provided in paragraph 16 above). I applied Equation 2 to each cell of the table above.

19. Finally, I calculated the conservation energy value (ΔG_i^{stat}) for at least one position i (and, in this case, each position i) in the MSA from the binomial probabilities referenced in paragraph 18 using the equation recited in claims 1 and 10. The plot below of these values reveals that some positions show conservation energy values close to zero and others show conservation energy values that differ to a greater degree from zero. These values quantitatively capture the degree of conservation of at least one side (and, in this case, each site) in the MSA.



20. Chemical polymers (*e.g.*, unstructured peptides) exist that have one or more amino acid positions but that do not contain protein.

Signed:____________________ Dated: 4/20/05

Name: Rama Ranganathan

Title: Associate Professor